THE VETERINARY JOURNAL

Editors:

T. DALLING, M.A., M.R.C.V.S.

J. McCUNN, M.R.C.V.S., L.R.C.P.

DECEMBER, 1945

EDITORIAL

HOW GOES THE FUTURE?

We are approaching the end of a momentous year, a year which the dispassionate historians of the future may recognise and acknowledge as a supreme turning point in evolution of mankind. It has been marked by what we all hope will be the final overthrow of the powers of evil men and also by man's greatest scientific triumph, the utilisation of atomic power. The future well-being of the whole world depends upon man's reaction at the present time. Can our differences in outlook and action be so adjusted that we may dwell together in peace and war with all its terror and waste shall be no more, or shall we tread the road to an inevitable doom?

Undoubtedly we are on the threshold of a future which, as yet, no one can forecast with certainty. There are those who say that the matter is of simple solution; all that is necessary is to disarm, put on one side the works of war, and all will be well. No doubt if all men were of the same mould or pattern this would be all that was required. Disarmament is an ideal, and idealists are apt to gaze through rose-tinted spectacles and pay little regard to practical things. Disarmament, if it is not universal, if it does not come from the spirit, and if there are not means at hand to enforce its obligations, is useless; indeed, it may be highly dangerous and foster the things which its supporters would wish to avoid.

There has been ample proof of the fallacy of such actions in the inter-war period, and we and our allies have suffered accordingly. Let us hope that a solution may be found, and rapidly, for there is much to do, and real progress cannot be made in the peaceful arts as long as the threat of strife hangs over an anxious world.

XUM

What part can veterinary science play in the solution of these problems? The answer is : It has a fundamental part, for the happiness and comfort of man largely depends upon the health and quality of his animals. A properly balanced system of animal husbandry means ample supplies of animal food products and a high state of fertility of the soil. When these two factors are assured there is food in abundance for all, and well-fed men are happy men. On the other hand, if there are shortages or want, man is discontented, he is apt to be jealous of his neighbour, we hear of the " haves " and the " have nots," and the seeds of war are set.

Therefore veterinary surgeons have a worthy task to do. They must exert every effort to enhance their competence, by making full use of all the knowledge which is at present to hand, and, above all, they must foster the spirit of inquiry and research. The art and science of the veterinary surgeon knows no bounds, and is for ever advancing. New vistas are opening every day, for knowledge, fundamental and specialised, is being steadily uncovered. It is the veterinary surgeon's duty to keep abreast of the times and so interpret these things that they may be made available at the earliest possible moment. Research and more research is a prime necessity. No occasion should be lost to bring the claims and benefits of veterinary research to the attention of institutions, societies, governments, and even private individuals. Money spent on research will pay big dividends. The profession can supply the men, men who, in virtue of their outlook and training, are competent to perform the tasks envisaged. All that is still needed are the facilities, so that our young men may set to work. If these are forthcoming great things are ahead which will bring untold benefit and reward to the world and help to draw nearer that time of peace and plenty for which we all yearn. Scientists from all over the world should be encouraged to meet, pool their knowledge, and work in harmony for the common good. Let us see that science is used for man's benefit and not for his destruction. In this we have grave responsibilities.

Christmas is drawing near, the time when man mellows to his neighbour; let us try and extend that spirit so that it will overflow into the years to come and spread amongst the nations, and that men, and especially those of science, may work for the common good.

APPOINTMENT

JAMES CARMICHAEL ESQ., M.R.C.V.S., Dip.Bact., has recently joined the technical staff of May & Baker, Ltd., Dagenham, with whom he will be closely identified in the study of veterinary research problems.

For a number of years Dr. Carmichael has been engaged in veterinary research in Uganda, in the course of which he directed important laboratory and field work on a number of subjects, including the evaluation of the diamidine series of drugs, and phenanthridinium compounds in babesiasis and trypanosomiasis.

General Articles

AN INFECTIOUS ENTERITIS OF YOUNG TURKEYS ASSOCIATED WITH COCHLOSOMA SP.

By JOHN G. CAMPBELL,
Department of Poultry Diseases, Royal (Dick) Veterinary College, Edinburgh.

THE relationship of the protozoan flagellate *Hexamita* species to an infectious catarrhal enteritis affecting young turkeys has been the object of an investigation by Hinshaw, McNeil and Kofoid (1938a and 1938b). These workers have reasonably concluded that *Hexamita* species is pathogenic to young turkeys, as it has been found in large numbers constantly associated with bowel lesions, and has proved infective to healthy poults when administered orally. Other flagellates were detected in the bowel contents, notably *Trichomonas* and *Chilomastix*, and amœbæ were also present, but all these were ruled out as causative agents.

No mention of the genus *Cochlosoma* is made in these papers, and as the protozoal and bacteria inhabiting the intestines of the affected turkeys were carefully noted in these American outbreaks, it was presumably not present.

Kimura (1934) and Travis (1938) have both studied *Cochlosoma* species in ducks, magpies and robins. McNeil and Hinshaw (1942), have reported *Cochlosoma* sp. in the intestines of turkeys, but remark that the significance of this parasite is not known.

The purpose of the present paper is to record the discovery of *Cochlosoma* sp. in very large numbers in the intestines of turkeys which were affected with a condition clinically and pathologically indistinguishable from infectious catarrhal enteritis due to *Hexamita* sp. (Hexamitiasis).

The outbreak occurred in early summer, on a large turkey farm in the North-East of Scotland. Young birds were invariably affected, the critical age being from two to ten weeks old. The symptoms reported, and later verified by a visit to the premises, were initially an intense thirst, followed by the development of a watery, frothy diarrhœa, depression, ruffled plumage, drooping head, closed eyes, loss of appetite, weakness, coma and death (Fig. 1). The sick birds congregated about the source of heat and were disinclined to move, but, when forced to, walked with a stilted gait.

The disease appeared to be extremely infectious, spreading rapidly through the pens, so that in a fortnight practically the whole of the young stock, 900 in all, had died. Death usually occurred within 2-3 days of the appearance of symptoms.

Naturally, with such alarming losses, advice was sought, and reports were received from four different sources, two suggesting dietetic errors, one "chilling," and one coccidiosis. Upon visiting the farm, it was obvious that the diet was not to be blamed, and generally the conditions were good, save in two respects —neither the food nor water bowls were protected by a wire cage, so that the young birds were walking in them and soiling the contents with their droppings— and the isolation of young from old stock was by no means perfect.

In all, twenty-eight poults were submitted to post-mortem examination, a number of these being brought alive to the laboratory in order to obtain fresh material.

Examination showed the birds to be in poor condition. The crops were

XUM

empty; some of the poults had nasal catarrh and slight pulmonary congestion, but the most marked lesions were in the upper digestive tract, the duodenum and jejunum exhibiting a catarrhal enteritis. The bowel contents were watery, and in parts yellow and frothy.

The intestinal tract, as a whole, was atonic, and one of the most characteristic lesions was the presence of dilatations, or bullæ, in the wall (Fig. 2). These bullæ contained yellowish froth, and the mucosa was congested. The cæca appeared to be normal in the majority of birds examined, although two showed a definite congestion of the mucous membrane. The gall-bladders were found constantly to be somewhat distended, and there was an œdematous condition of the connective tissue in this region.

Coccidia were not detected in any of the poults, and no bacteria of any significance were isolated from the intestines, liver or heart blood. Microscopical examination of the heart blood also gave negative results. The possibility of a filterable virus infection was considered, but transmission experiments could not be undertaken because of lack of suitable birds. This, however, is not considered a serious omission, as the nature of the disease does not suggest a virus infection.

Microscopical examination of the intestinal contents revealed little or nothing in birds which had been dead for 12 or more hours. In freshly dead specimens, however, the bowel contents were found to be swarming with a pyriform flagellate, swimming jerkily and with a decided spiral movement. These were especially numerous in the yellow fluid contents of the bullæ, and direct smears stained and examined microscopically showed them to be present almost to the exclusion of any other protozoa.

Attempts were made to cultivate these flagellates in Hartley's broth containing 2½ per cent. glucose over a slope of inspissated ox serum, the whole sealed with paraffin, but in every case bacteria proliferated to such an extent that the protozoa died. In one set of attempts, gentian violet in various dilutions was added to a suspension of intestinal contents until the protozoa were seen to be almost motionless, and then cultures made from this treated suspension, in the hope that the majority of the bacteria would be killed, whilst leaving the flagellates viable, but this, too, was unsuccessful.

These parasites, which appear to belong to the genus *Cochlosoma*, were present in every fresh case examined. A few *Trichomonads* and a few *Hexamita* were also usually detectable, but by far the majority of organisms present were identified as *Cochlosoma* species.

Description of the Flagellate

With dark field illumination, these organisms appear as small periwinkle-shaped flagellates, swimming jerkily with a spiral rotation. A group of flagellæ arises from near the anterior extremity, and trail backwards along the side of the body. The nigrosin dark ground method of outlining the organisms and flagellæ was tried, but in such preparations the flagellæ appeared matted together and it was impossible to estimate the number.

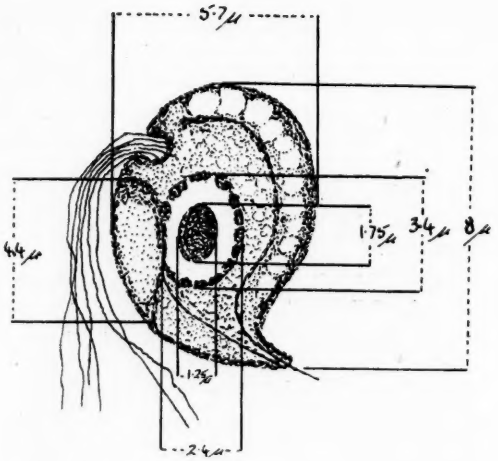
Smears from the bowel contents, stained with May-Grünwald-Giemsa combination, show the main structure of this organism quite well. The drawing (Diag. 1) is based on the study of many individual organisms in several stained preparations. With the May-Grünwald-Giemsa method of staining, the cytoplasm is basophilic and vacuolated, the central oval nucleus has a prominent peripheral

XUM

FIG. 1. Typically affected turkey poults, as seen in the field.

FIG. 2. The intestinal tract from a typical case. Note the congested bullæ along the length of the bowel.

XUM



DIAG. 1. Drawing of *Cochlosoma* sp. based on the study of stained smears and dark-ground illumination preparations. The dimensions are average figures from fifty individual specimens.

FIG. 3. Transverse section of affected small intestine. The mucosa is heavily infiltrated, and is sloughing in parts. The débris in the crypts consists of shed epithelial cells and *Cochlosoma* sp. X80. H. & E.

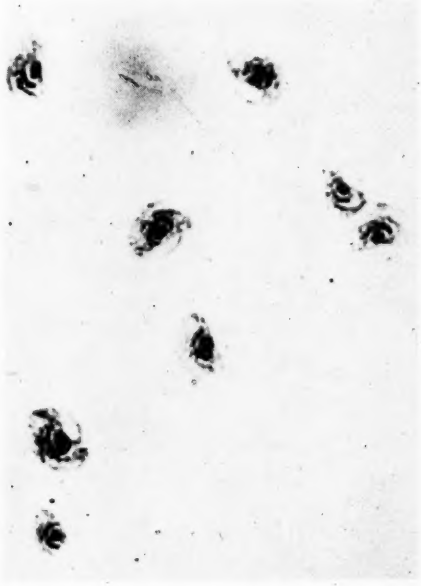


FIG. 4. *Cochlosoma* sp. X1500. May-Grünwald-Giemsa stain.

FIG. 5. *Cochlosoma* sp., with desquamated intestinal epithelial cells, for comparison of size. X1500. May-Grünwald-Giemsa.

XUM

zone of deeply staining chromatin granules, denser on the dorsal aspect. This thick nuclear membrane encloses a clear hyaline space, in the centre of which is a bright red, oval karyosome. The axostyle is clearly visible as a purple-staining curved structure arising from a primary blepharoplast which is situated antero-ventrally. It curves to the right, i.e., dorsally, following the greater curvature of the cell and passes to the posterior end, where it projects about 2 μ from the cytoplasm. The chromatic basal rod or costa has a similar staining reaction. It arises from a quaternary blepharoplast situated to the left of the nucleus, and curves round to run adjacent to the axostyle. It does not, however, project beyond the curved tail-piece of the organism.

The ventral aspect of the flagellate is provided with a large sucker, appearing in stained preparations as a clear oval area situated in the middle third of the left side. The flagellæ (assumed in the diagram to be six in number, in conformity with all known species of *Cochlosoma*) arise from a small pit at the anterior extremity of the ventral surface. Most of these features, apart from the flagellæ, can be distinguished in the photographs of the organisms.

Histopathology

Fig. 3 shows that the most prominent features of the affected intestine are (a) intense cellular infiltration into the mucosa, and (b) necrosis and desquamation of the epithelium of the villi. Large numbers of the parasite are present in the crypts.

Discussion

The genus *Cochlosoma* is a flagellate belonging to the family Trichomonadidæ and order Protomonadida (Wenyon, 1926). It is of interest to the protozoologist in that it appears to be an intermediate form between the trichomonad flagellates of the Monozoa and the Giardia of the Diplozoa.

For example, the sucker, the two axial fibrils and the centrally disposed nucleus are giardia-like, whilst the flagellæ, arising from a blepharoplast at the anterior end of the body, the presence of a single nucleus, and the axial fibrils, which appear to be homologous with the costa and axostyle, are trichomonad characters.

The only evidence brought forward regarding the pathogenicity of *Cochlosoma* is by Kotlán, who created the generic name in 1923. In one duck he noticed the intestinal wall was swollen and catarrhal at the point where a mass of flagellates were attached, and that the intestinal contents were mixed with blood. Travis (1938) was unable to detect any pathological condition in the infected birds he studied, and he remarks that controlled experiments are needed to demonstrate the effect of these flagellates on their hosts.

Although such experiments were unfortunately not possible in this instance, all the available evidence points to the pathogenicity of *Cochlosoma* to turkey poults under suitable conditions.

Diagnosis can only be made with certainty when freshly dead birds are examined, and in any future outbreak it is to be hoped that transmission experiments will be undertaken.

The diagram shows the main morphology of the flagellate, as seen in preparations stained with May-Grünwald-Giemsa. It differs mainly from previously published diagrams by Kotlán and Travis in the representation of the nuclear structure (these authors show a simple nucleus, and a very small karyosome) and in

XUM

the presence of a small, well defined, cup-shaped cavity at the antero-ventral extremity, from which the flagellæ appear to arise. Possibly the primary blepharoplast is associated with this structure.

The diagrams given by these authors also represent the organisms from the ventral aspect. As will be observed from the photo-micrographs appended (Figs. 4 and 5), the organism, being flattened laterally, is always seen from the side in fixed and stained films. This is difficult to reconcile with Travis's description of the organisms as "broadly ovoidal in dorsal or ventral view . . . from a lateral view the body is slender."

It will be seen from Table I. that the dimensions of the *Cochlosoma* sp. found associated with this enteritis of young turkeys agree closely with the figures given by Travis and Kotlán for *C. anatis* and *C. rostratum* respectively. According to Travis, these two are probably synonymous, and so, apart from the slight discrepancy in figures for the nuclear dimensions, which may be due to a difference in interpretation of structure, it appears justifiable to conclude that the *Cochlosoma* species described in this paper is *C. anatis* (Syn. *rostratum*).

TABLE I.

Showing dimensions in microns of *Cochlosoma* sp. from turkeys, as compared with *C. anatis* and *C. rostratum* from North American ducks.

	C. anatis (N. Amer. Ducks). Travis.	*C. rostratum* (N. Amer. Ducks). Kotlán.	*Cochlosoma sp.* (Turkeys, Scotland). —
Length.—			
Mean ...	8.4	8.1	8.0
Range ...	6.5-11.5	6.1-10.0	—
Width.—			
Mean ...	4.9	5.5	5.7
Range ...	4.0-6.0	3.9-6.7	5.5-6.0
Nucleus.—			
Mean ...	2.1	1.4	3.4 x 2.4 (oval).
Range ...	1.2-3.0	1.2-1.7	3.0 x 2.0 - 4.0 x 2.5 (Prominent Karyosome 1.75 x 1.25 μ.)
Length of Sucker.—			
Mean ...	4.8	4.2	4.4
Range ...	4.5-5.0	3.3-4.4	4.0-6.0

Summary

An acute infectious disease of young turkeys indistinguishable from infectious catarrhal enteritis due to *Hexamita* sp. (Hexamitiasis) is recorded, and is believed in this instance to be due to a flagellate protozoan belonging to the family Trichomanadidæ and genus *Cochlosoma* Kotlán. The main features of the morphology of the flagellate are recorded, and its dimensions compared with some other known species.

It is emphasised that a diagnosis of this disease can only be made with perfectly fresh warm material, as the flagellates die and disappear within a few hours of the death of the host. It was impossible, at the time of the outbreak, to obtain young, healthy poults for transmission experiments, but it is hoped that such experiments will be undertaken at some future date.

REFERENCES.

Hinshaw, W. R., McNeil, E., and Kofoid, C. A. (1938a): "The Relationship of Hexamita sp. to an Enteritis of Turkey Poults." *Cornell Vet.*, 28: 281.

Hinshaw, W. R., McNeil, E., and Kofoid, C. A. (1938b): "The Presence and Distribution of Hexamita sp. in Turkeys in California." *Journ. Amer. Vet. Med. Ass.*, 96, (N.S.46) No. 3: 160.

Kimura, G. G. (1934): "Cochlosoma rostratum sp. nov: an Intestinal Flagellate of Domesticated Ducks." *Trans. Amer. Micr. Soc.*, 53: 102-116.

Kotlán, A. (1923): Cited by Travis.

McNeil, E., and Hinshaw, W. R. (1942): "Cochlosoma rostratum from the Turkey." *Journ. Parasit.*, 28: 349.

Travis, B. V. (1938): "A synopsis of the Flagellate Genus Cochlosoma. Kotlán, with the Description of Two New Species." *Journ. Parasit.*, 24: 343.

Wenyon, C. M. (1926): "Protozoology." Vol. I, p. 681. London: Bailliére, Tindall & Cox.

ACTIVE IMMUNISATION AS AGAINST PASSIVE SEGREGATION WITH REFERENCE TO TUBERCULOSIS

By SYDNEY GORDON TIPPETT, M.B.(Lond.),

Formerly Medical Superintendent, Nordrach-upon-Mendip Sanatorium.

THE medical profession is at last taking a definite interest in this urgent problem of how best to control tuberculosis among our cattle. There have been letters in the medical press over the failure of the Ministry of Health to safeguard the public from being poisoned by milk. There is a strong demand for a clean and safe milk supply.

Up to the present the Ministry of Health and the Medical Research Council, together with the Ministry of Agriculture and their Animal Research Council, have all got tired of this problem of the control of tuberculosis. It is to be hoped that with the coming of a new Government they will insist on these public health bodies attacking the whole problem afresh.

Recently the Medical Research Council has been again urged to try and solve this problem of immunity with reference to tuberculosis. This is quite as much a problem for the Ministry of Agriculture to find the right answer. Though the problem may seem insoluble, I am quite convinced that it is not so, and I can conceive of no greater benefit which the medical and veterinary professions could confer on mankind, and in the animal world, too, than that these bodies should succeed in solving it. The amount of economic loss alone, due to tuberculosis, in any one year is enormous, and who can measure the total of human suffering and loss?

To make clear what is required, the following illustrates what is needed.

In the case of a human being, any attack of an infectious disease is followed either by death or recovery. When recovery follows, then substances described as antibodies can be demonstrated in the blood. This fact has been employed to good use.

Animals can be injected with certain germs and made to produce antibodies in their blood. These antibodies can then be used to treat cases of disease occurring in human beings. In many instances bacterial poisons, or even the bacteria themselves, having had their virulence suitably demonstrated, can be injected into human beings, and thus produce immunity to the disease in question. But, with regard to tuberculosis, it has not been found possible to do anything of the kind. Why is tuberculosis an exception to the rules governing practically all other infections? This is probably the most important question confronting modern medicine in this country, and is equally important in the veterinary work of the Ministry of Agriculture.

Unfortunately little interest has been taken in trying to find out to what is resistance to infection in the case of tuberculosis due. No help can be obtained

from the Ministry of Health nor the Medical Research Council about this question re resistance to infection, nor can any light be given by all the various societies interested in the tuberculosis question. A certain amount of work on the liability to tuberculosis has been carried out by the American workers, Kallman and Reisner. This was reviewed in a medical journal[1] : " Thus, while genetic influences are clearly potent in determining whether or not manifest tuberculosis is contracted, they are much more potent in determining the resistance of the individual to the established disease. It is a remarkable illustration of this finding that the chances of death from pulmonary tuberculosis of a person with the disease who has a healthy identical twin are practically nil."

To compare once again with work carried out on human beings, a Dr. Ward[2] (late hon. secretary to the Joint Tuberculosis Council) has stated that 75 per cent. of children are born with a high resistance to infection, and *a certain percentage could not contract clinical tuberculosis under any circumstances.*

Though the Ministry of Health has no idea to what this natural resistance is due, yet it does exist, and, of course, the same is true of cattle.

Very little work has been done in this country, yet the problem cannot be insoluble. It only requires sufficient work to elucidate it.

Turning to this question of active immunisation *versus* passive segregation, one can see why our Ministry of Agriculture take up their present attitude, as at any rate it saves them the trouble of trying to find some way of building up the resistance of our cattle against infection by the tubercle bacillus. The acid test is, does it answer? And here the practical experience of farmers show that this policy is definitely fraught with grave dangers, as it will in the end lead to our breeding a race of beasts which will have no immunity at all. If such animals then come in contact with infection they will die like flies. Already there are rumours that certain herds which have been tubercle free for a long time have sudden flare-ups of disease and consequent losses. There must be records known to the Ministry of herds which have been free according to the test for a long time, and then the whole picture has changed within the period between two tests to one showing numerous reactors. Take the evidence of the late Professor Calmette, of the Pasteur Institute, who strongly criticised our present methods for the prevention of tuberculosis[3] :

" The folly of the British attitude is shown by the fact that even if it were possible to eradicate tuberculosis completely by the enforcement of some draconic system of segregation of bacilli carriers, the only way to maintain on the British Isles this hypothecated absence of tuberculosis infection would be to cut off all relations with the rest of the world.

" The less virulent infections which cause tuberculin allergy (and immunity) in most adults would then become impossible, and after some time every British subject would be as receptive of tuberculosis as were the former inhabitants of countries where tuberculosis did not exist. It is Utopian to expect that tuberculosis can be eradicated by measures of hygiene only, even if they were practicable on a large scale. The only measure that answers the purpose is preventive vaccination.

" Killed vaccines, such as those against typhoid, cholera, plague or undulant fever, give rise to only a transient immunity, only the presence in the human body of living tubercle bacilli, whether they be virulent or avirulent, such as B.C.G., renders it immune to tuberculosis."

XUM

We could not have stronger criticism of our present methods.

In Denmark, years ago, it was planned to prevent tuberculosis among cattle by taking away the calves as soon as they were born, so that they never came in contact with any animal with tuberculosis. It was hoped that this method would give the Danes herds free from all risk of tuberculosis.

It was found that these herds were very liable to become infected, as their natural inherited resistance was lost, and once they were brought in contact with T.B. germs they were soon infected with virulent disease.

The farmer and the public do not get a fair deal. The farmer is doing his work under conditions which are in so many cases all wrong. He needs a proper water supply from the mains, and he needs electric light and power and proper cowsheds, and his fields want proper drainage.

Now, once a cow is found to be tubulous it should be illegal to take that animal and send it to a market, where it will be sold to some other farmer, and so spread infection to some other herd.

The price paid by the Government when an animal has to be slaughtered is too low, and is the cause why the farmer tries to get rid of the animal by selling it. A fair price should be paid to the farmer. At present the public is getting milk with living germs of tuberculosis in it in many areas. There are statements in the Press to-day that in this country some 40 per cent. of the cattle are infected with tuberculosis. An enquiry by the People's League of Health[4] disclosed that "A proportion of raw market milk contains living tubercle bacilli to an average of 6.7 per cent. In England and Wales about 6 per cent. of all deaths from tuberculosis are caused by the bovine type of bacillus. About 2,000 deaths in England and Wales, mostly in children, occur annually from this cause. At least 4,000 fresh cases of bovine infection develop each year, an immense amount of suffering, invalidity, and often permanent deformity being thus caused by this bacillus."

How much improvement has taken place since that report was drawn up over thirteen years ago. The work of Lurie[5] on rabbits is most interesting. He found that in experimenting with greatly reduced intensity of exposure, the results clearly paralleled the human findings. Certain rabbits of high genetic resistance acquired an infection which healed completely, while others of a less genetic resistance similarly exposed to the same low intensity of contagion developed fatal tuberculosis. In regard to human beings, it follows from the above that human pulmonary tuberculosis is a disease influenced to a notable extent by inborn inherited constitution, and influenced to a greater degree in regard to its behaviour when once contracted than in regard to whether it is contracted at all.

It is clear that the present policy of the Ministry of Agriculture is wrong. It is a case for careful experiments to be carried out to learn how best to build up resistance against infection.

One of the main reasons why our cows so easily become tubulous is that they are made to produce far more milk than a cow would do in its natural or wild state.

Cattle breeders in the U.S.A. have found that the control of tuberculosis is mainly a matter of prevention in new-born calves, and the adult cattle may be disregarded.

In a medical journal[6] one sees : " About 40 per cent. of the cattle slaughtered in abattoirs in Northern Ireland are found to have naked-eye lesions of tuber-

XUM

culosis, and about 6 per cent. of samples of unheated milk taken from supplies delivered to households in Belfast are infected with virulent tubercle bacilli."

Would a similar survey in this country show the same appalling state of affairs?

The real problem to-day in this country is to find out the best way to build up resistance against infection in those animals that are known to have a very poor resistance. As our public health authorities have not managed to find out to what resistance is due, nor how we can build it up, we must go back to nature and use her own work. Research work carried out for human beings should be available for animals, and work carried out for animals should be used for the benefit of human beings.

We know that a high degree of resistance against T.B. infection exists in certain children and in certain cattle. We also know that if a child or an animal has not been infected by the T.B., then if given a dose of tuberculin there is no reaction.

The work of Lurie shows that if an animal of high resistance is infected by the T.B. it should be able to destroy that infection. It must be possible to transfer some part of an animal's high degree of resistance to another animal with a low degree of resistance till such time as our health authorities can show how this resistance is built up.

In the case of human beings it has been found possible to give a dose of tuberculin by the mouth and obtain a reaction with it. It is also possible to rub tuberculin into the skin of a human being and again obtain a reaction. No doubt it will be found possible to give a dose of tuberculin to an animal by the mouth and still obtain a reaction.

A calf should be treated so that it will possess a very high degree of resistance against T.B. infection in the ordinary way. It may be found possible to give a dose of B.C.G. by the mouth to a calf and then, as soon as it reacts to tuberculin, to treat the calf by giving it tuberculin by the mouth until all the B.C.G. germs are killed. It should be possible to produce active immunisation by small doses of a bovine tubercle bacillus in the case of a calf, and then later kill the T.B. germs by tuberculin. It is still true, as stated by Robert Koch, that tuberculin will kill T.B. germs in their earliest stage.

So far it has never been considered worth while to treat an animal for tuberculosis. This is wrong, and if we will only heed what is to-day known about the disease in human beings, we can see a reason for this. In the *B.M.J.* of October 6, 1945, it is stated: "As a recovered case rarely breaks down, a patient whose disease is actually cured probably has such a high resistance to disease that he will never suffer again from this scourge of mankind."

If this is what is known to take place in a human being, why not in a calf or cow? The position would be at once improved if in future we were to breed only from those cattle that were known to have a very high degree of resistance against tuberculosis, and if, in addition to this, we ceased to overwork our cows by making them into mere machines for milk production.

REFERENCES.

1. *B.M.J.*, October 14, 1944.
2. "Tuberculosis." By Dr. Ward. *Clinical Journal*, October, 1944.
3. Article in *Medical Press and Circular*, December 22, 1943. Calmette's criticism of our present methods for the prevention of Tuberculosis now proved to be fully justified.
4. "Tuberculosis, Its Cure and Prevention." By Gordon Tippett.
5. *B.M.J.*, October 14, 1944.
6. *B.M.J.*, August, 1945.

VITAMINS IN ANIMAL HEALTH

By RUTH ALLCROFT

Veterinary Laboratory, Ministry of Agriculture, Weybridge.

PART II.—THE WATER-SOLUBLE VITAMINS

(*Continued from page* 232.)

Vitamin B Complex

The term "B complex" arises from the fact that a number of water-soluble substances with related physiological properties were originally regarded as a single vitamin, termed "water-soluble B," in distinction to "fat-soluble A" and the antiscorbutic "vitamin C." As research proceeded it became apparent that "water-soluble B" comprised at least two components, "heat-labile B_1" and "heat-stable B_2." The antineuritic factor was then separately identified as thiamine and retained the designation B_1, while at the same time it was recognised that "B_2" was not a single substance, and a variety of imperfectly recognised entities came to be referred to as the "B_2 complex." This latter term includes factors subsequently identified as riboflavin, nicotinic acid, pyridoxine, pantothenic acid, biotin, *p*-aminobenzoic acid, folic acid, inositol and choline.

It has recently been suggested that the whole B group might be divided into three sub-groups, (a) the three members, thiamine, riboflavin, and nicotinic acid, which are associated with more or less clearly defined deficiency diseases; (b) pyridoxine, pantothenic acid, biotin, *p*-aminobenzoic acid, and folic acid, which have been shown to be essential growth factors for some micro-organisms and essential dietary factors for some animals; (c) inositol, choline, and perhaps methionine, which are lipotropic factors and control fat deposition in the liver and certain other organs.

Most of the members of the vitamin B complex have certain characters in common which justify their consideration as a group. They appear to be universally distributed in living matter and enter into the make-up of all organisms, from the single-celled bacteria and yeasts to the most highly developed vertebrates. Many of the members, certainly thiamine, riboflavin, nicotinic acid and pyridoxine, form part of complex enzyme systems, concerned with the metabolic reactions of living cells. These systems consist of a molecule of which the vitamin is a part, coupled with a protein carrier, the whole exhibiting enzymic activity or participating in the activation of an enzyme. There is also a general tendency for the various members of the group to be associated together, and sources which are rich in one of the B vitamins are also likely to be rich in the rest, while poor sources of one are not likely to be good sources of others.

It is well known that species differences exist with respect to animal requirements of the B vitamins, as well as to requirements for vitamins in other categories. As early as 1915, Theiler, Green and Viljoen showed that cattle could live for extended periods (13 months) on a diet of polished rice with a small moiety of poor quality oat straw autoclaved at 130° C., and suggested that ruminants could synthesise their own vitamin requirements in virtue of the extensive flora of the alimentary tract. Although only two vitamins were clearly recognised at that time, the anti-scurvy vitamin and the anti-beriberi vitamin, reconsideration of the original dietary shows it to be grossly deficient in all the vitamins known to-day. In so far as the B group is concerned, later work has shown that the apparent independence of ruminants is mainly due to synthesis by the microflora of the rumen. Using fistulated animals, ruminal syntheses of

XUM

thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid and biotin have been demonstrated specifically. Intestinal bacteria, with few exceptions, have also been found to produce thiamine, riboflavin, nicotinic acid and biotin, and bacterial synthesis in general is now known to include eight members of the B complex. In animals with simple stomachs, however, any vitamins formed by bacterial synthesis in the alimentary tract tend to occur beyond the absorptive areas and to be evacuated in the fæces. Such animals are therefore dependent on dietary supplies, unless, like the rat under certain experimental conditions, they develop the habit of coprophagia; or, like the rabbit, indulge in faecal refection as a normal custom of the species.

Vitamin B_1

This vitamin is known also as thiamine, or aneurin, and is sometimes still referred to as the antineuritic or anti-beriberi vitamin. The latter designations arose from the fact that it was recognised quite early that beriberi and avian polyneuritis resulted from deficiency of what was thought to be a single substance present in the pericarpal layers of rice, but absent in the endosperm. It was later realised that in addition to its antineuritic properties, the substance which protected against beriberi also contained various other factors now included within the term "B complex."

The empirical formula of thiamine hydrochloride is $C_{12}H_{17}ON_4SCl\ HCl$; it contains a pyrimidine group combined with a thiazole group. The vitamin can now be synthesised in the pure state and is prepared commercially more easily by synthesis than by isolation from natural sources. One International Unit of vitamin $B_1 = 3\mu g.$ of thiamine-hydrochloride = one U.S. Pharmacopœia Unit.

Occurrence. The natural precursors of thiamine and the reactions leading to its synthesis in the plant are unknown. It is widely distributed throughout the plant and animal kingdom, but plants are much better sources than animal tissues. The richest natural sources are yeasts and the pericarpal layers and embryo of cereal grains and legumes; the endosperm of grains contains very little. Although most animal tissues contain thiamine, the actual amount is small and varies greatly in different species. For example, pork muscle contains about eight times as much as beef muscle. The vitamin is not stored to any significant extent in the animal body, but the highest relative concentrations are found in the liver, kidneys, heart, muscles and brain of animals having an adequate diet. The amounts in the tissues of animals which do not synthesise it are influenced by the thiamine in the diet.

Physiology. Within the last eight years it has been shown that thiamine (aneurin) is directly concerned with intermediary carbohydrate metabolism. In the form of its pyrophosphoric acid ester it functions in the tissues as a co-enzyme (co-carboxylase) which catalyses the decarboxylation of pyruvic acid, an intermediary degradation product of carbohydrates. It has been suggested that it can also be concerned directly or indirectly in numerous other reactions in which it is possible that decarboxylation or carboxylation may enter at some stage as an essential feature.

It has been demonstrated that fat has a "sparing" action on thiamine Rats on a B_1 deficient diet have continued to grow without typical symptoms of thiamine deficiency when large amounts of fat were included in the diet. Conversely, fat synthesis has been found to be impaired by thiamine deficiency. Proteins have also been shown to have a thiamine-sparing action.

Deficiency. Although recent researches have not altered the belief that human beriberi is primarily due to a deficiency in the diet of thiamine, the actual number of clear-cut clinical experiments on record in which adult beriberi was treated by this compound alone, without other dietary alteration, is not large. The diets on which the disease develops are usually deficient in other factors besides B_1, and some of the symptoms grouped under the heading "beriberi" may be due to other deficiencies as well.

There are two well-marked forms of the disease in the adult. In the "dry type" there is great muscular wasting, dysæsthesia of the skin, and, finally, paralysis of the legs, and in some cases of the arms, diaphragm and intercostal muscles. There is an associated degeneration of the peripheral nerves, involving both motor and sensory fibres. In the "wet type" of the disease the most marked symptom is œdema, involving the limbs and trunk and effusion of serous fluid into body cavities. There is marked dilation of the heart, particularly of the right side, together with congestion of the liver and abdominal viscera. Heart failure, often sudden in onset, is common and the mortality is high. With regard to the ætiology of beriberi, it was shown about the beginning of the present century to be associated with the eating of highly milled rice from which the germ and pericarp were removed, and it could be cured or prevented by restoring the milling products to the diet or by eating unpolished rice. It has been repeatedly mentioned by clinical workers on human beriberi that an ill-balanced diet involving deficiencies of proteins and inorganic salts and an excessive allowance of carbohydrate may play a part in the production of the disease. It has been suggested that the œdema of "wet" beriberi may be due to shortage of protein.

More detailed investigations of vitamin B_1 deficiency in humans have shown that the known anatomical lesions are polyneuropathy, the ophthalmoplegia of Wernicke's syndrome and circulatory disturbances.

In a well-controlled experiment with human subjects it was shown that a characteristic neurasthenic syndrome could be induced as a result of partial thiamine deficiency, and it has been reported that deprivation in man affects first of all the emotions and the tone of the nervous system.

The symptoms of B_1 deficiency in rats resemble very closely the symptoms in humans, but there is also a marked influence upon the growth of young animals. In pigeons, chickens and turkeys the symptoms are similar. There is a decrease in appetite, progressive development of general paralysis, and, in the later stages, head retraction, followed by death. B_1 deficiency in poultry is rarely observed under practical conditions, since most poultry foods contain ample amounts of the vitamin.

Symptoms of thiamine deficiency, including anorexia, vomiting and impairment of growth, have been produced in pigs by feeding experimental diets, but there is no evidence that pigs on ordinary rations ever develop the syndrome. The thiamine requirement of young pigs has been shown to be related to the fat content of the diet; animals on a low level of fat showed evidence of thiamine depletion much sooner than those on a medium or high level of fat.

It has now been shown that Chastek paralysis in foxes is due to a thiamine deficiency caused by destruction of the vitamin in the food by some factor present in raw fish. The disease was observed in foxes kept on farms where uncooked fish was included in the ration at a level of 10 per cent. or more. The mortality was reported to be especially high among nursing pups. The clinical manifesta-

XUM

tion of the disorder is characterised by hyperæsthesia, anorexia, weakness, ataxia, spastic paralysis, and rapid death 48 to 72 hours after onset of the symptoms. Post-mortem examination revealed bilateral vascular lesions in the brain located principally in the paraventricular grey matter. These lesions resemble those of Wernicke's hæmorrhagic encephalopathy in man and pigeons. It was found that the disease could be prevented by adding large amounts of thiamine to the food or by cooking the fish before giving it to the foxes.

Paralysis of the hind limbs has been reported in dogs fed a diet of raw meat and biscuits with a vitamin supplement. When the meat was cooked the paralysis was relieved, and it is suggested that there is some substance in raw meat as well as in raw fish which destroys or antagonises vitamin B_1.

Symptoms of thiamine deficiency can also be produced in cats by feeding an exclusive diet of raw carp or raw herring.

Vitamin B_2

Riboflavin, or vitamin B_2, has the empirical formula $C_{17}H_{20}N_4O_6$, and its constitutional designation is 6, 7-dimethyl-9 (*d*-1′-ribityl) iso-alloxazine. It belongs to a group of coloured water-soluble, naturally occurring substances called lyochromes which are present in a variety of plant and animal tissues. They were named by affixing the termination "flavin" (to indicate the yellow colour of the pigment) to the source name. Thus, the flavin present in milk was named "lactoflavin," that in eggs "ovoflavin," in liver "hepatoflavin," in urine "uroflavin," and in yeast "zymoflavin." Since in lactoflavin the sugar radical is ribose, the term "lactoflavin" was changed to "riboflavin," and because all the biologically active flavins are chemically identical, they are all known now as "riboflavin," and the terms "lactoflavin," "ovoflavin," "hepatoflavin," etc., have been discarded.

In cells and cell products, such as milk, riboflavin exists in the free state or is bound with protein, when it is called "flavoprotein." The yellow fluorescent pigment formed by the tubercle bacillus appears to be identical with riboflavin.

Since it is more expensive to isolate pure riboflavin from natural sources than it is to synthesise it, synthetic vitamin B_2 is generally used clinically.

An International Unit of riboflavin has not yet been established. A unit in common use is the B.S. Rat Unit (Bourquin-Sherman Unit), which is defined as the daily amount of riboflavin required by rats to insure proper growth, i.e., 10 g. increase in body weight per week for two to four weeks. This is equivalent to 2-3 μg. riboflavin.

One Cornell Unit of riboflavin = 1 μg. riboflavin, defined by the growth effect on chicks.

Occurrence. Riboflavin occurs in nearly all plant and animal tissues. The richest sources are certain anærobic fermentation bacteria, such as butyric acid bacteria, yeasts, animal organs, including liver, kidney, spleen and lean meat, egg-white, milk and certain vegetables and fruits.

The destruction by light of riboflavin in foods has been found to be rapid. For example, sliced bread may lose riboflavin by being exposed to light under ordinary conditions, and increase in temperature facilitates destruction.

Physiology. Riboflavin phosphoric acid forms with a specific protein the yellow oxidation enzyme flavoprotein (Warburg's "yellow enzyme") which plays an important part in tissue respiration. There are seven flavoprotein enzymes known and they form part of complex oxidation-reduction systems acting as dehydrogenases and take part in the oxidation of carbohydrates, amino-acids, lactic

XUM

acid and aldehydes. The vitamin is therefore probably required for the growth and maintenance of all tissues.

There are no special storage organs for riboflavin in the animal, although a certain level appears to be maintained in the various tissues and relatively large amounts are found in the liver and kidneys. Administration of large amounts of riboflavin does not increase the riboflavin content of the liver to any appreciable extent, while, on the other hand, the organs of animals which die from B_2 deficiency still contain considerable amounts, approximately one-third of the normal level. It is excreted predominantly in the fæces and to a smaller extent in the urine; during deficiency none is found in the urine.

It has been reported that riboflavin plays an important part in economy of food utilisation, both for growth and for maintenance, since in riboflavin deficiency the intermediate products of metabolism appear to be wasted through incomplete combustion.

Deficiency. The first manifestation of riboflavin deficiency is retardation, followed by complete cessation of growth. The more specific symptoms vary to some extent from one species to another. In rats the deficiency is characterised by a specific involvement of the ectodermal organs, such as the skin and the eyes, as well as by cessation of growth. Progressive alopecia is one of the chief symptoms; it is localised in certain areas, such as the back between the shoulders, the lines joining the eyes and ears, and finally the chest. The fur may appear moist and matted, and in advanced cases the eyes are sometimes surrounded by a spectacle-like loss of hair, while the eyelids are often stuck together with a serous reddish fluid. If riboflavin is not added to the diet, the animals finally die from general exhaustion. Chronic riboflavin deficiency has frequently been found to be accompanied by pediculosis. This was at first regarded as accidental and unspecific, but pediculosis has never been reported in rats receiving a vitamin B-free diet supplemented with thiamine and riboflavin, even when they were moribund from a B_6 (pyridoxine) deficiency. Also, therapeutic administration of riboflavin to B_2-deficient rats with pediculosis has resulted in complete disappearance of the pediculi, often accompanied by replacement of the diseased patches with new fur. The practical implications of these findings with special reference to skin parasites in man have not yet been investigated.

Probably the earliest and most specific lesions which develop in riboflavin deficient rats are ocular changes, involving diffuse corneal vascularisation and opacity. It has been stated that cataract also occurs, but the evidence for the rôle of riboflavin deficiency in the production of this lesion is very conflicting.

In man the clinical picture of riboflavin deficiency consists of (1) ocular lesions, consisting usually of a vascularising keratitis with photophobia, dimness of vision, severe injection of the vessels of the fornix and sclera, burning of the eyes, lachrymation, and, in severe cases, opacities of the cornea; (2) oral lesions, consisting usually of linear fissures in the angles of the mouth, a reddened, shiny, denuded appearance of the lower lip, and a flattening of the papillæ of the tongue, which becomes magenta red in colour; and (3) dermal lesions, consisting usually of seborrhœic accumulations in the folds of the skin, especially in the nasolabial folds, around the eyelids, and on the ears. In some cases the seborrhœic lesions may be extensive and involve other regions of the body.

Chicks are apparently very sensitive to a deficiency of riboflavin. In addition to stunted growth, a rapid and severe paralysis of the legs has been reported

XUM

to be characteristic of the acute deficiency, while a slowly developing disability of the feet and legs, the so-called "curled toe paralysis," occurs as the result of a chronic deficiency. A lesion at the junction of the upper beak and the flesh (notched beak) is also reported as a sign of riboflavin deficiency in chicks.

On low riboflavin diets egg production is markedly reduced, and eggs which fail to hatch show abnormalities in the embryos, which include degeneration of the Wolffian bodies, deformed down, œdema and dwarfing.

The symptoms of riboflavin deficiency in turkey poults differ from those in the chick. A dermatitis or skin inflammation is reported to appear in young birds after about eight days on a deficient diet, when the vent becomes encrusted, inflamed and excoriated. Growth is retarded and ceases completely about the seventeenth day, and deaths begin to occur about the twenty-first day.

Under intensive rearing conditions and when birds are kept closely confined the foodstuffs used for poultry may not contain sufficient riboflavin to meet the minimum requirements of the chick or turkey during the first few weeks of life, unless green foods or other good sources, such as dried skim-milk or dried buttermilk, are supplied. The minimum riboflavin requirement of the growing chick and poult varies with their age, and has been reported to be about 1,300 μg. per pound of food for the chick and about 1,600 μg. for the poult. An adequate supply for chicks, poults and ducks for the first four or five weeks has been put at about 1,670 μg. per pound of food.

Riboflavin deficiency has been produced experimentally in pigs; young animals on a riboflavin-deficient diet failed to grow normally, vomited frequently, had severe diarrhœa, developed a rough skin and a peculiar gait, had considerable exudate about the eyes, and died. It is suggested that the minimum daily requirement of riboflavin for young growing pigs is from 1 to 3 mg. per 100 pounds live weight. In pigs, and apparently also in dogs that have reached a severe stage of riboflavin deficiency, a sudden fatal collapse often occurs, with signs of vagotonia and drop in body temperature; post-mortem examination reveals a "yellow" fatty liver, and degenerative changes in the central nervous system have been described. Corneal changes, as well as scaly dermatoses similar to those encountered in rats, have also been observed in dogs and pigs.

It is unlikely, however, that a spontaneous uncomplicated riboflavin deficiency would occur in pigs and dogs, and it is much more probable that it would be complicated with deficiencies of other members of the B group.

Nicotinic Acid

Although nicotinic acid was known as a chemical entity in 1867, its possible rôle in nutrition was not considered until 1911, when Funk found it among substances isolated from rice polishings in the search for the antineuritic vitamin. It failed, however, to cure polyneuritis in pigeons and its vitamin nature was not further investigated until 1935, when it was found to be an essential part of one of the coenzyme systems. The need for nicotinic acid in the growth of *Staphylococcus aureus* was demonstrated also, and it was later found to be required by *Corynebacterium diphtheria, Bacillus proteus,* lactic acid bacteria and certain protozoa and fungi.

Then in 1937 Elvehjem and his co-workers, who had been studying the effect of various liver extracts on experimental blacktongue in dogs, found that nicotinic acid would cure canine blacktongue and that nicotinic acid amide could be

XUM

isolated from a blacktongue-curative fraction of liver. Later in the same year the effectiveness of nicotinic acid in the treatment of human pellagra was demonstrated by several independent groups of workers, and the compound was shown to be the same as the pellagra-preventive or P.P. factor originally postulated by Goldberger as necessary for avoidance of human pellagra.

The name "niacin" has been put forward as a synonym for nicotinic acid and is largely used in the United States. The empirical formula for nicotinic acid is $C_6H_5O_2N$. Its constitutional designation is pyridine-3-carboxylic acid. There appears to be no need for an international standard, since both the acid and the amide are well-known crystalline compounds which can be used on the weight basis.

Occurrence. Nicotinic acid is reported to occur in all living cells in small amounts. The richest sources are liver, yeast, red muscle meats, kidney, and wheat germ and bran; vegetables, some cereals and milk contain small amounts, while fatty meats are poor sources. Even the richest sources, however, contain only 5 to 20 mg. of nicotinic acid per 100 g. net-weight portions.

Physiology. It is now well established that nicotinic acid amide or nicotinamide is a constituent of two coenzymes, namely (1) coenzyme I (or cozymase, or co-dehydrogenase I), in which it is present as diphosphopyridine nucleotide, and (2) coenzyme II (or co-dehydrogenase II), where is occurs as triphosphopyridine nucleotide. The nicotinamide part of these two coenzymes undergoes reduction and oxidation and functions in the cell as a hydrogen transporter and controls intracellular oxidations. The concentrations of these coenzymes in liver and muscles have been reported to be lowered in cases of nicotinic acid deficiency in man, dogs and pigs.

Synthetic nicotinic acid is readily absorbed from the intestinal tract, and presumably the more complex derivatives which occur in foods are also easily absorbed. There are no special storage organs for the acid or any of its derivatives since it is present in most of the tissues, but the greatest amounts are found in the liver: for a water-soluble vitamin its storage is fairly good, as it takes from 30 to 40 days, and sometimes longer, to deplete the reserves of a dog sufficiently to allow the development of blacktongue.

In man the acid is excreted in the urine as trigonelline, nicotinuric acid, free nicotinic acid, and as N′-methylnicotinamide derivatives; dogs excrete only trigonelline and nicotinuric acid and no nicotinic acid or its amide. It is reported that rabbits cannot synthesise trigonelline from nicotinic acid.

Deficiency. It is well established that inadequate nicotinic acid is the principal dietary defect leading to clinical pellagra, but since a pure vitamin deficiency in man is rare it is most likely that the disease is the result of a multiple deficiency. This would explain to some extent the great variations in symptoms and lesions which occur in this disease, and why, although the cardinal symptoms are cured by nicotinic acid, it is often necessary to give other members of the B complex as well as vitamins A and C, together with proteins and mineral salts.

It is reported that there are six types of dermatitis seen in pellagra: (1) The typical dermatitis over the exposed surfaces, especially those parts of the body which are exposed to sunlight or to friction; (2) sebaceous-gland changes about the face; (3) thickening and pigmentation over the bony prominences; (4) perineal and genital lesions; (5) lesions of the lips and mucocutaneous surfaces at the corners of the mouth; and (6) changes in the superficial structures of the

XUM

eye. It is probable that (2), (4), (5) and (6) are signs of riboflavin deficiency, though they commonly occur in pellagra. Disturbances of the gastro-intestinal tract are observed in many cases, while in later stages of the disease mental disorders and lesions of the central nervous system occur. Exposure to sunlight greatly aggravates existing symptoms and causes development of further symptoms. It has been suggested that the acute inflammation of the skin, the gastrointestinal disturbances and the dementia which develop after a pellagrin is exposed to direct sunlight, resemble the symptoms which have been observed in acute porphyrin intoxication, and some investigators have found an increased excretion of coproporphyrin I in both urine and fæces of pellagrins.

In dogs, nicotinic acid deficiency results in the occurrence of blacktongue, which is manifested by lassitude, loss of appetite, occasional vomiting and characteristic lesions of the mouth. The mucous membranes of the cheeks, gums and tongue become reddened and inflamed, sometimes showing extensive purplish-red areas where congestion is most marked. The inner surfaces of cheeks and lips may be covered with pustules and ulcers. Constipation is often noted at the onset of the disease and diarrhœa develops in the later stages. If the disease has not progressed too far treatment with nicotinic acid or liver extract effects recovery, otherwise death occurs. It has been shown that administration of 0.23 mg. nicotinic acid per pound body-weight will cure the symptoms of blacktongue. It has been suggested that there may be some danger in administering large doses of nicotinic acid in the presence of a deficiency of other factors of the B complex. The diet should be adjusted to include foods such fresh liver, dried yeast, beef muscle, wheat germ and milk, which are good sources of nicotinic acid and other vitamins of the B complex.

The spontaneous occurrence of blacktongue has been observed most often in areas where the dog's ration is somewhat similar in character to the diets producing pellagra in man, that is, where the ration consists largely of products such as corn meal, salt pork, cow-peas and sweet potatoes.

It has been reported that a condition similar to canine blacktongue occurs in cats and responds to the same treatment.

There is some evidence that nicotinic acid is not essential in the diet of the horse, since balance studies showed that the output of nicotinic acid in the urine and fæces was considerably greater than the intake on the low nicotinic acid diet which was used. This suggests that the vitamin is synthesized in the gut of the horse.

When pigs were maintained on a Goldberger or pellagra-producing corn diet, symptoms developed which resembled those of nicotinic acid deficiency. The chief symptoms were refusal of food, severe diarrhœa, loss of hair, development of a dirty yellow skin, a severe scabby dermatitis and a muscular difficulty in the use of the hind legs. Intramuscular injection of nicotinic acid, followed by daily supplements in the food, cured the condition.

It has been suggested that necrotic enteritis of pigs is not a primary infection due to *Salmonella suipestifer,* but a secondary complication developing as a result of a deficiency of nicotinic acid. Successful use of the vitamin in the treatment of this condition in pigs has been reported by some workers, while others have found that administration had no effect on the course of the infection in pigs maintained on diets either low or adequate in nicotinic acid.

XUM

Vitamin B_6 (Pyridoxine)

This vitamin is most commonly known as pyridoxine, but is sometimes referred to as "adermin," the "anti-acrodynia rat factor" or the "rat anti-dermatitis factor." Chick and Copping were the first to produce good evidence for the existence of this member of the B complex and they called the vitamin "factor Y." In 1943 György established the difference between the "rat-pellagra preventive factor" and B_2 and called the new vitamin "B_6". Later he introduced the term "pyridoxine" for this factor, thus reducing much of the confusion caused by the use of so many different names for the same vitamin.

Pyridoxine was isolated in the pure state in 1938 and synthesized a year later. Its empirical formula is $C_8H_{11}O_3N$. Its constitutional designation is 3-hydroxy-4, 5-di(hydroxy-methyl)-2-methyl-pyridine.

Occurrence. Pyridoxine has been found in all foods so far assayed and appears to be widely distributed over the entire plant and animal kingdom. Yeast, wheat germ and rice polishings are especially good sources, seeds and legumes fairly good, while fruits, vegetables and milk are fairly poor sources. Among animal products, muscle, liver, kidney and heart are all good sources, and so is fish liver and muscle.

Physiology. Little is known about the physiological action of pyridoxine, but it has been suggested that it is concerned in protein metabolism, particularly in the synthesis of fat from protein and in tryptophane metabolism. Recent work indicates that pyridoxal, a derivative of pyridoxine, acts as a coenzyme of amino-acid decarboxylases.

Deficiency. The pathology of pyridoxine has been studied almost entirely in the rat, which is the only animal that shows the characteristic dermatitis. Recent reports, however, indicate that it is also an essential for other animals, such as the chick, dog, pigeon and pig; the evidence that it is an essential human requirement is very meagre and inconclusive.

The dermatitis which occurs in pyridoxine deficient rats is stated to be quite characteristic and can be distinguished from the dermatitis which occurs in deficiencies of other members of the vitamin B complex. The paws, snout and ears are involved in an acute erythema and œdema, followed by desquamation which may finally lead to ulceration, especially on the paws, where rubbing becomes a factor. This specific dermatitis was called "acrodynia" by György, but some investigators have reported that pyridoxine deficiency in the rat is not always accompanied by acrodynia. However, all observers agree that the skin of the trunk is not demonstrably affected by the deficiency. Cessation of growth has also been reported.

The occurrence of convulsions or fits of an epileptiform nature has been reported in pigs, dogs and rats maintained on pyridoxine-deficient diets. The animals become abnormally excited and any extra stimulus induces fits. No dermatitis, however, has been observed in pigs and dogs.

The development of a microcytic hypochromic anæmia has been reported to occur in pyridoxine deficient dogs and pigs. The hæmoglobin level and the number of red cells decreased progressively, the hæmoglobin relatively faster than the red cells. Addition of pyridoxine caused a great reticulocyte response with rapid increase in hæmoglobin and red cells. It is suggested that pyridoxine is essential for hæmoglobin formation, although just what role it plays is at present unknown. After nearly a year on diets free of pyridoxine, dogs have

XUM

been observed to develop cardiac embarrassment and degenerative changes in the peripheral nerves.

The symptoms of pyridoxine deficiency in chicks have been described as slow growth, depressed appetite and inefficient utilization of food. Their requirement has been put at about 300 μg. per 100 g. of food. This vitamin has also been reported to be necessary for egg production in pullets. In turkeys, deficiency symptoms are described as loss of appetite, poor growth, apathy, hyper-excitability when disturbed, and convulsions.

Since pyridoxine is so widely distributed in natural foodstuffs it is unlikely that deficiencies of this vitamin would occur under normal conditions.

Very little is known concerning the action or requirements of this vitamin in man, but it has been reported that complete or considerable relief from the nausea and vomiting of pregnancy has been obtained by its administration. A combination of pyridoxine and thiamine was found to be superior to pyridoxine alone and much better results were obtained in first pregnancies than in later pregnancies.

Pantothenic Acid

The existence of pantothenic acid was first noted in 1933 by Williams and his colleagues as a "yeast-growth substance." It subsequently proved to be the "filtrate factor" component of yeast and liver, previously known to be necessary for the growth and well-being of the rat and for prevention of dermatitis in chicks. The name pantothenic acid was suggested because of its apparently universal occurrence in living matter. The empirical formula is $C_9H_{17}O_5N$ and the structural designation is α,γ-dihydroxy-β, β-dimethyl-butyryl-β-alanine. It is commercially available in the form of its crystalline synthetic calcium or sodium salt. Pantothenic acid concentrates, for example from rice bran, are also marketed.

Occurrence. The vitamin occurs in all types of animal tissues and seems to be present universally in protoplasm. Liver and kidney are the richest known animal sources. It is produced by various moulds, micro-organisms and green plants; rice bran and wheat bran are especially rich sources.

Physiology. Very little is known about the physiology of pantothenic acid in man and animals. It has been reported that it is partly destroyed in the body and is constantly excreted in the urine. It is known to be an essential growth factor for certain micro-organisms, for example, lactic acid and propionic acid bacteria, streptococci and pneumococci. It has been suggested that pantothenic acid is concerned in certain aspects of carbohydrate metabolism.

Deficiency. The vitamin has been shown to be essential for various animals, including rats, mice, chickens, pigs and dogs.

In rats the most significant lesion, preventable by pantothenic acid, that has been described, is a fatal hæmorrhagic cortical necrosis of the adrenal glands. Other manifestations of the deficiency are alopecia, scaliness and thickening of the skin with eventual ulcerations, which occur about the nose and head and over the shoulders, flanks and abdomen of both rats and mice. Inflammation and encrustation of the nose, mouth and eyes have also been reported to occur in the rat and chick.

Chicks on a pantothenic acid deficient diet develop a specific dermatitis. The skin epithelium becomes keratinised and a dry sloughing occurs. Growth and feathering are retarded, and the down and feathers become ragged in

appearance. Encrustations occur about the eyes, the corners of the mouth, and the areas between the toes. Besides the dermatitis, certain lesions have been reported to occur in the spinal cord, characterised by degeneration of the myelinated fibres.

It has been shown that adult fowls need pantothenic acid for hatchability and reproduction but not for egg production. The hatchability of eggs has been observed to drop from 70 per cent. to 3 per cent. when the vitamin was removed from the hen's diet. It has been suggested that naturally occurring deficiency disorders exist in poultry, since it has been shown that chicks fed on a diet containing 75 per cent. whole maize, supplemented with casein and with other vitamins, developed typical dermatitis. It has also been reported that feeding extra pantothenic acid to hens already on a good commercial diet increased the hatchability of the eggs 30 per cent. above the controls.

Pigs on a pantothenic acid deficient diet show loss of appetite, weight and hair, develop diarrhœa (often bloody), weakness and inco-ordination of the legs, showing a characteristic "goose-stepping" gait. Degeneration of the myelinated fibres and extensive ulceration of the intestinal tract have also been reported.

The first manifestations of experimental deficiency in puppies were observed to be an erratic lowered appetite and a decrease in urinary excretion of the vitamin accompanied by reduction in growth. Collapse occurred after two months. Spasticity was observed in the hindquarters during the last week of the deficiency. The only gross pathological changes observed were fatty livers.

There appears to be some controversy as to whether pantothenic acid is involved directly, indirectly, or not at all, in the greying of the hair or nutritional achromotrichia which has been observed in rats, mice and foxes on experimental diets deficient in pantothenic acid. It seems probable that more than one dietary deficiency or set of factors can lead to development of grey hair, among which may be included lack of pantothenic acid.

Biotin or Vitamin H

In 1901 Wildiers described the stimulating effect of small amounts of an extract of yeast on the growth of certain strains of yeast. He gave the name "bios" to this substance. It was later shown to be multiple in nature and was fractionated into bios I, bios IIa, bios IIb and other components. The bios IIb fraction was shown to be identical with a substance which Kögl isolated in 1936 from eggs and which he called "biotin." Later it was observed that biotin had properties similar to an extract called "coenzyme R," which had growth and respiration promoting effects for *Rhizobium trifolii*, a legume nodule organism.

In 1927 Boas observed that when large amounts of egg white were included in the diet, rats developed an eczema-like dermatitis accompanied by loss of hair. She found that this condition could be cured by a factor present in liver, which she called "protective factor X." This factor was later isolated by György, who named it "vitamin H." In 1940 biotin, coenzyme R and vitamin H were all shown to be identical.

Investigation of the factor present in egg white, which produced specific lesions in rats, showed that the effects were the result of an induced biotin deficiency due to the formation of a non-absorbable complex between biotin and a constituent of the egg white. This substance has recently been isolated and

XUM

shown to be a protein-carbohydrate complex to which the name "avidin" has been given because of its avidity for biotin.

Biotin appears to be extraordinarily active and can be detected by the yeast growth method in as low a concentration as 1 part in 10,000,000,000.

The empirical formula of biotin is $C_{10}H_{16}O_3N_2S$ and its structural designation is 2'keto-3, 4-imidazolido-2-tetrahydrothiophene-*n*-valeric acid. It has recently been synthesised.

Occurrence. By the use of very sensitive microbiological assay methods, biotin has been found to be very widely distributed and it appears to be a common constitutent of every type of living cell. Perhaps because of its very high physiological activity, biotin is present in very low concentrations in tissues. For example liver, which is one of the richest sources, contains less than 0.0001 per cent. Such low concentrations make it necessary to use very large amounts of material for isolation procedures. It has been stated that in order to produce 1 g. of crystalline biotin methyl ester, 360 tons of yeast or 1½ million eggs would have to be used. Amongst the richest sources of the vitamin are liver, kidney, yeast, pancreas, milk and eggs; it is present in relatively high concentrations in the seeds of plants and increases during germination.

Physiology. Very little indeed is known concerning the physiological action of this vitamin. A relationship has been suggested between biotin and fat metabolism, since fatty infiltration of the livers and an increase in the body fat of rats has been obtained by administration of the vitamin. By contrast rats which had suffered a severe biotin deficiency were found to possess almost no stores of body fat.

Deficiency. So far no naturally occurring biotin deficiencies have been reported in farm animals, but experimental deficiencies have been produced in some species.

The symptoms in the rat may be briefly described as a generalized, pruritic exfoliative dermatitis, coupled with seborrhœa, alopecia, poor growth, and an abnormal "kangaroo-like" posture. A typical denudation around the eyes occurs and this is known as the "spectacle eye" condition.

In chicks and in turkeys deficiency results in a specific dermatitis. The bottoms of the feet become rough and calloused and, in more severe cases, encrustations with hæmorrhagic cracks appear. In addition, lesions occur in the corners of the mouth and around the beak, and the eyelids become swollen and stick together. Biotin appears to be necessary for the normal embryonic development of hen eggs.

An experimental biotin deficiency has been produced in man and it has been suggested that there are certain similarities between some of the symptoms observed in biotin-deficient rats and those occurring in "pink disease" in children and seborrhœic dermatitis in man.

Folic Acid

In 1941 Mitchell and his colleagues reported the isolation from spinach of a vitamin-like substance which was found to be abundant in other green leaves also. The name "folic acid" was proposed because of its occurrence in leaves. It has since been shown to be present in liver, kidney and yeast, and to be a growth factor for certain micro-organisms. There is evidence that the anæmia and leucopenia caused by administering sulphonamides to rats can

be cured by folic acid and it is thought to be identical with a substance called vitamin Bc. This term was applied to the factor in extracts of liver which was required by the chick when fed a purified diet for the prevention of an anæmia of the hyperchromic macrocytic type. Folic acid also appears to be closely related to xanthopterin (a substance related to the purines) which has been reported to cure anæmia in trout, and to vitamin M, a factor present in liver and yeast which cures anæmia and leucopenia in monkeys fed on a synthetic diet.

p-Aminobenzoic Acid

In 1940 Woods found that the antibacterial action of sulphanilamide could be inhibited by certain yeast extracts and showed that the factor present in the extracts was identical with *p*-aminobenzoic acid. He put forward the theory that suphanilamide competes with *p*-aminobenzoic acid at the surface of an enzyme which is essential for growth, and suggested that normally *p*-aminobenzoic acid is a constituent of this hypothetical enzyme, but can be displaced by sulphanilamide and that when this happens the enzyme can no longer function and growth ceases. It has since been shown that *p*-aminobenzoic acid is an essential metabolite for all organisms that are inhibited by sulphanilamide.

The acid appears to be as widely distributed in nature as most of the factors of the B complex. Yeast, from which it was first isolated, appears to be the richest source. It has been known as a chemical compound, of course, since its synthesis by Fischer in 1863. Its presence in most tissues, fluids and elimination products of the body has been demonstrated.

The growth-inhibiting action of sulphonamides for certain micro-organisms has been used to study the function of some of the intestinal microflora. It has been shown that sulphonamides such as sulphaguanidine and succinyl sulphathiazole, which are absorbed to a very limited extent from the gut and are therefore good intestinal antiseptics, when fed in fairly large amounts over a long period of time to rats and chickens, cause symptoms resembling deficiencies of certain members of the vitamin B complex. Some of the harmful effects could be counteracted with liver extract, vitamin K, biotin and with *p*-aminobenzoic acid. Further work showed that the sulphonamides destroyed or inhibited the microflora which synthesized these vitamin B factors. During these investigations it was noticed that a greying of the hair occurred in rats fed on a diet containing sulphaguanidine, supplemented with thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, biotin, choline and inositol; this condition could be cured by a folic acid concentrate. It was therefore suggested that nutritional achromatrichia was dependent on at least three factors, namely pantothenic acid, folic acid and *p*-aminobenzoic acid, the latter substance entering into the picture only in so far as it influences the intestinal flora which synthesise the factor or factors concerned.

It has been demonstrated that *p*-aminobenzoic acid can exert a detoxifying action in the body for certain substances. Symptoms of hydroquinone poisoning were shown to be overcome by its oral administration and it was found to be very effective in rats as a detoxicant for lethal doses of pentavalent arsenical compounds used in the treatment of trypanosomiasis; the trypanocidal action of the arsenic compounds were not inhibited. Protective action against the toxic effects of antimony compounds, particularly against the pentavalent

XUM

"Stibosan," have also been reported, but no reduction in the toxicity of "Mapharsen" for mice has been found.

Although information on the role of *p*-aminobenzoic acid in bacterial metabolism is rapidly accumulating, little is known concerning its function in mammalian physiology and authorities differ in regard to its vitamin action. It has, however, been reported to be a dietary factor for chicken growth and feathering, and to stimulate folic acid production by the intestinal flora of chicks.

Inositol, Choline and Related Substances

Inositol and choline, together with certain other substances such as methionine and betaine, are sometimes included in the B complex, although it is doubtful whether they should be designated as vitamins.

Inositol has been shown to be identical with bios I (c.f. section on Biotin) and is hexahydroxy-cyclohexane. It occurs as a normal cell constituent in practically all plant and animal tissues. In plants it occurs chiefly in the form of phytin, the calcium and magnesium salt of inositol hexaphosphate. It has been shown to be a growth factor for certain micro-organisms. Very little is known about the physiology of inositol but like choline and methionine it is a lipotropic factor, that is, it prevents the accumulation of fat in the liver and aids the normal distribution of fat in the liver tissues. Inositol deficiency in rats has been reported to cause the development of a special type of fatty liver containing large amounts of cholesterol.

Low choline diets, especially when the fat intake is high and protein low, lead to retardation of fat "turn over" in the liver. During chronic deficiencies in the dog and rat, a diffuse cirrhosis has been reported. In addition to the development of fatty livers, acute choline deficiency in young rats causes a severe hæmorrhagic condition of the kidneys and other tissues, and an involution of the thymus gland.

In hens a choline deficiency has been reported to cause decreased or discontinued egg production, increased mortality and absorption of egg yolks, and in turkeys and chicks it appears to be among the factors involved in the prevention of perosis or "slipped tendon disease."

It has recently been established the choline, methionine and betaine function as methylating agents in the body. Choline is a normal constituent of ordinary mixed diets, and is a component of lecithin and other phospholipids present in animal foodstuffs.

Other Suspected Factors of the B Complex

In addition to the components already mentioned, others are suspected. For example, a factor influencing feather development in birds has been referred to as B_{10} and an obscure growth factor for chicks has been provisionally termed B_{11}. It is customary to assign a B number until identity is established, although this procedure is often confusing to the general reader since numbers often fall away later or become merged together.

Vitamin C, the Antiscorbutic Vitamin

Scurvy, the typical manifestation of vitamin C deficiency, has been known for centuries and it was common knowledge that the disease occurred especially among sailors, after deprivation of fresh foodstuffs for long periods. About three hundred years ago it was realised that it could be prevented and rapidly

XUM

cured by providing fresh vegetables and fruits. Between 1918 and 1925 Zilva attempted the concentration of the antiscorbutic substance from lemon and obtained almost pure ascorbic acid and established the important basic properties of the vitamin.

In 1932 it was shown that the antiscorbutic substance in lemon juice was identical with "hexuronic acid" $C_6H_8O_6$, which Szent-Györgyi isolated in 1928 from adrenal cortex and oranges. The name "hexuronic acid" was then dropped and the term "ascorbic acid" adopted. The name "cevitamic acid" was given to the vitamin by the American Medical Association, but this, too, has been discarded in favour of "ascorbic acid." The acid is a derivative of gulose, being the enolic form of 3- keto- *l*-gulofuranolactone with the empirical formula $C_6H_8O_6$. The international standard for vitamin C is such that one International Unit of vitamin C = 0.05 mg. of pure ascorbic acid = one U.S. Pharmacœpia Unit.

Occurrence. The vitamin is widely distributed over the plant and animal kingdom; it is present to some extent in all living plant cells and animal tissues contain small but definite amounts. Citrus fruits, berries and green vegetables are excellent sources, and large amounts are found in all actively growing parts of higher plants. When the active metabolic processes cease or are greatly reduced as in seeds, the amount of ascorbic acid is greatly diminished or is entirely absent. As soon as germination begins, however, the presence of the vitamin in considerable amounts can be demonstrated. Although, in general, animal tissues contain only small amounts, certain organs, especially the endocrine glands, contain larger quantities; the adrenal gland is especially rich in the vitamin.

Physiology. In spite of the wide distribution of vitamin C, little is known of the mechanism of its formation in plants and animals; it has been suggested that ascorbic acid may be formed from certain acids of related structure, such as glucuronic or galacturonic acid, but there is also some evidence from experiments on starving rats that the precursor of the vitamin is of endogenous origin and independent of carbohydrate intake. It has recently been suggested, but not confirmed that the presence of traces of manganese is necessary for the successful synthesis of ascorbic acid from mannose and to a lesser extent from galactose and glucose in plant and in animal tissues.

Ascorbic acid is easily destroyed by heat or by exposure to the air. The factor responsible for this destruction is ascorbic acid oxidase, which occurs in plant materials along with ascorbic acid and becomes more active on heating. From the standpoint of human nutrition this is a most important point because of loss of the vitamin in cooking and preserving processes, but in animal nutrition it is probably not of serious consequence.

The metabolism and general physiology of vitamin C are still very imperfectly understood. It appears that all animals and plants need the vitamin, but apart from a few exceptions they are able to synthesize it. The exceptions, which are known to require an external supply, are man, the other primates, the guinea pig and a few micro-organisms. The site of formation of ascorbic acid in animals which synthesise it is not known, but it is thought to be in the intestinal tract.

It has been shown that dairy cows maintained for three to four years on a low vitamin C diet, continued to excrete vitamin C. There was no decrease from

XUM

normal in the vitamin content of the blood plasma and milk during this period. Balance experiments on these animals showed that the output of the vitamin far exceeded the intake, and that the output was highest in the fæces, next highest in the milk, and was extremely small in the urine. From these observations it is suggested that synthesis in the dairy cow might take place along the gastro-intestinal tract, somewhere beyond the rumen, where it has been demonstrated that vitamin C is destroyed. On the other hand, however, it has been shown that when organisms were taken from various parts of the gastro-intestinal tract of ruminants and grown in media prepared from the contents of the organ from which they were taken, very little or no vitamin C was found to be present, although good growths were obtained.

In simple-stomached animals absorption of ascorbic acid occurs principally in the small intestine. When vitamin C is taken by mouth the blood plasma level rises to a mixiumum within one to two hours, but the increase is only temporary, as the vitamin is transported by the blood throughout the entire organism and excess amounts are excreted in the urine. Normal human blood plasma contains about 1.2 mg. per 100 ml. There are no real storage organs for this vitamin, and the species that cannot synthesise it are dependent on a regular supply in the food. Cows' milk contains on the average about 22 mg. per litre, but the level varies somewhat with the season and the highest amount has been found in the late summer or early autumn. Pasteurisation of milk does not seriously decrease the amount of ascorbic acid and the loss is not as great as that caused by exposure of milk to sunlight for several hours.

The most obvious property of ascorbic acid is its capacity for reversible oxidation and reduction, and much speculation and experimental work has arisen from the idea of correlating this property with the mechanism of the physiological action of the vitamin. Amongst the various functions suggested for vitamin C there has been a considerable amount of evidence in support of the view that vitamin C is of major importance in the detoxification processes of the body. It has been shown that injections of diphtheria toxin, or certain other toxic materials, may cause a marked depletion of the vitamin from the tissues, and that animals whose tissues are moderately or severely depleted of their vitamin C reserves are subject to greater injury. There is evidence that in cases of active tuberculosis and other infections there is an increased demand for vitamin C, and it has been reported that the anaphylactic effect of horse serum in guinea pigs has been greatly reduced by administering vitamin C.

A close relationship between vitamin C and various hormones has been suggested, since during avitaminosis a decreased hormone secretion was observed from those glands which normally contain high concentrations of the vitamin, for example, adrenal, pituitary, pancreas and thyroid. The breeding performance of horses has been reported to be related to the blood ascorbic acid values; good breeding mares had a significantly higher value than difficult breeders. Other work suggests that the breeding efficiency of some bulls with low fertility could be improved by subcutaneous injections of ascorbic acid, but the observations require confirmation.

Deficiency. As has already been stated, the only animals known to require an external supply of vitamin C are man, monkeys, and guinea pigs. The pathology of ascorbic acid deficiency in susceptible animals and in the naturally

XUM

occurring deficiency in man, i.e., in cases of scurvy, may be described very simply as the failure of formation and maintenance of intercellular materials; the morphological changes occurring in avitaminosis C are almost entirely restricted to the supporting tissues of mesenchymal origin.

The symptoms of severe scurvy in guinea pigs are, briefly, tenderness and swelling of the joints and gums; the latter become spongy and bleed easily. Later the teeth become loose, solid food is refused, and death may occur shortly afterwards. Post-mortem lesions show close analogy with those characteristic of the human disease. Hæmorrhages may occur in any position, but are most frequent in the limbs. Rarefaction of the long bones is almost invariably present, and these frequently show fracture in the neighbourhood of the junction of shaft and epiphysis; the ribs are swollen and often fractured at the junction of bone with cartilage. The teeth are loose and can easily be removed. One of the earliest symptoms of C deficiency is a change in the structure of the teeth, which can be detected when the other scorbutic symptoms are so slight as to be almost unrecognisable. These changes in tooth structure have been used as a means of estimating ascorbic acid biologically.

As has already been mentioned, there is evidence that saturation of the tissues with vitamin C promotes healing of wounds and fractures of bones. The average optimal intake of human beings is about 50 to 100 mg. ascorbic acid daily. The requirements for guinea pigs are from 1 to 2 mg. daily.

Vitamin P

There is still considerable doubt as to the existence or significance of vitamin P, which is also known as citrin. About ten years ago the occurrence was reported by Dam in Copenhagen of a substance other than vitamin C which controlled hæmorrhages. He called it "Permeabilitäts vitamin," hence the name vitamin P. Since then there have been very conflicting reports concerning its action. It is thought that the vitamin is concerned with the maintenance of normal conditions in the walls of the small blood vessels and that its absence causes increased capillary fragility and permeability. The view has also been expressed that vitamin P may be required for the absorption and retention of ascorbic acid. A method involving the capillary resistance of guinea pigs has been used to assay various types of fruits and vegetables for vitamin P activity, and it has been found that fruits, especially citrus fruits, are the best sources. Extracts which exhibit vitamin P potency contain, besides other components, glucosides of eriodyctol and hesperitine. The presence of these substances has not yet been demonstrated in animal materials, such as milk, liver or kidney.

A state of vitamin P deficiency has been reported for the guinea pig and rat, and for man. In human subjects reported to be suffering from avitaminosis P, capillary resistance was decreased and vascular permeability increased. The clinical symptoms comprised hæmorrhagic conditions of the skin which could not be cured by administration of ascorbic acid.

The author wishes to acknowledge the help and criticism given by Miss A. M. Copping, of the Division of Nutrition, Lister Institute of Preventive Medicine, and by Dr. H. H. Green, of this laboratory at Weybridge.

XUM

Reviews

A History of Medicine. By Douglas Guthrie, M.D., F.R.C.S.(Ed.), F.R.S.E. Thomas Nelson & Sons, Ltd., Parkside Works, Edinburgh, 9. 30s. net.

In his preface the author quotes Mr. Winston Churchill: "The longer you can look back the further you can look forward." Truer words were never uttered. A history of medicine is the story of the evolution of the healing art, and as active participants in this art veterinary surgeons will find this book of great interest and delight. The art and science of medicine can be likened to a great and beautiful building, as yet unfinished, which has been built up stone by stone, sometimes by the aid of plans, other times at the whim of the builder and to no preconceived design. Doctors were the first professional men, functioning as magicians and medicine men, holding both spiritual and temporal power over their fellows, much as is the case in primitive races even to-day. The early records of these men are depicted in crude drawings, and the oldest picture of a medical man, no doubt more a magician than a doctor, is to be found on the wall of a cave in the Pyrenees. It is estimated that he lived about 15,000 B.C. The medical profession is, therefore, at least 16,000 years of age. These early practitioners depended for their success on their knowledge of magic and mystery; their knowledge of disease must have been of secondary account, yet it is strange that, with the crudest of instruments, they could perform successfully such delicate operations as trephining the human cranium. It would also appear that this particular operation was performed in various parts of the world at a time when there could have been no interchange of knowledge between such widely separated practitioners. Dr. Guthrie takes the reader from these times through all the ages until the present day. Through all these ages the seat of medical lore has migrated from place to place, according to the rise and fall of nations and empires: Mesopotamia, Egypt, then to the Mediterranean area, Greece, Ionian Islands, Montepelier, Italy Bologna, Salerno and Rome, to Europe, Britain and America. We read of places which have become familiar to all in the present conflict in Cos and Salerno. He takes in his review to the great men of medicine, Se Khetenanach and Inthotep, who were so renowned as to be later venerated as gods, and Hippocrates, to the giants of modern times, Lister, McEwan, Osler, etc. We read of the progress made in Mediæval and Renaissance times, of Harvey, Leonardi de Vinci, the dropping of superstition, witchcraft and the priesthood influences, and the change to a rational concept based primarily on a sound knowledge of anatomy. We see the birth of the modern scientific approach and the great advances made in surgery. He gives us pen and other pictures of great medical men of both the old and modern schools which are an inspiration to any young medical man.

Dr. Guthrie's book is not a formal history naming men and dates; it is a real, live story of the birth and growth of a science which will hold the reader from start to finish. It will be of particular interest to the veterinary surgeons in this country at the present time, when changes are suggested and even threatened in the system of veterinary education, for a knowledge of the past is essential to those who would plan the future.

The book is beautifully produced, almost in the best pre-war style, and the illustrations are in keeping with the general format.

XUM